CAGCGTCAGACGCAGGGCACTGAGAATGTGCGACAGCGCGCAACGATGAAGTAGCCCAGAGGGTCCCTTG GAAAATGAGGCCAGGGTCCCTGCTGCTGCTTGTTCTGCTGCTCGCCCTGTCCAGGAGCCTGCGGGGCAAA GAGTGTGCGTCTCCACCCTGTGAGTGTCACCAGGAGGACGACTTCAGAGTCACCTGCAAGGAGCTCCACC GAATCCCCAGCCTGCCGCCCAGCACCCAGACTCTGAAGCTCATCGAGACTCATCTGAAGACCATACCCAG TCTTGCATTTTCGAGTCTGCCCAATATTTCCAGGATCTATTTATCTATAGATGCAACTCTGCAGCGGCTG GAACCACATTCTTCTACAATTTGAGTAAAATGACTCACATAGAAATCCGGAACACCAGAAGCTTAACCT ATATAGACCCTGATGCCTTGACAGAGCTCCCCTTGCTCAAGTTTCTTGGCATTTTCAATACTGGACTTAG AATATTCCCTGACTTGACCAAAATTTATTCCACGGACATATTCTTTATACTTGAAATCACAGACAACCCT TACATGACTTCGGTCCCTGAAAACGCATTCCAGGGCCTATGCAATGAAACCTTGACCCTGAAACTGTACA ACAATGGATTTACTTCAGTCCAAGGACATGCTTTCAATGGAACAAAGCTGGATGCTGTTTACCTAAACAA GAATAAATACCTGACAGCTATAGACAACGATGCCTTTGGAGGAGTATACAGTGGACCAACTTTGCTAGAT GTGTCTTCCACCAGCGTCACTGCCCTTCCTTCCAAAGGCCTGGAGCACCTCAAAGAACTGATCGCAAAAG ACACCTGGACTCTCAAAAAGCTCCCGCTGTCGTTGAGTTTCCTCCACCTCACTCGGGCTGACCTCTCTTA  $\tt CCCGAGCCACTGCTGCGCTTTTAAGAACCAGAAGAAAATCAGGGGAATCCTGGAGTCTTTGATGTGTAAT$ GAGAGCAGTATCCGGAACCTTCGTCAAAGGAAATCAGTGAACATCTTGAGGGGTCCCATCTACCAGGAAT CTCTCACTATTACGTCTTCTTTGAAGAACAAGAGGATGAGGTCGTTGGTTTCGGCCAAGAGCTCAAAAAT CCTCAGGAAGAGACTCTCCAAGCCTTCGAGAGCCACTATGACTACACGGTGTGTGGGGACAACGAGGACA  ${\tt TGGTGTACCCCCAAGTCGGACGAGTTTAACCCCTGTGAAGATATCATGGGCTACAGGTTCCTGAGAAT}$  $\tt CGTGGTGTGTTGTCAGTCTGCTGGCTCTCCTGGGCAATATCTTCGTCCTGCTCATTCTGCTAACCAGC$  ${ t CACTACAAATTGACCGTGCCGCGGTTCCTCATGTGCAACTTGGCCTTTGCAGATTTCTGCATGGGGGTAT$ ACCTGCTTCTCATTGCCTCTGTAGACCTGTACACACACTCTGAGTACTACAACCACGCCATCGACTGGCA GACGGGCCCTGGGTGCAACACGGCTGGCTTCTTCACTGTTTTCGCCAGTGAGTTATCAGTGTACACACTG ACGGTCATCACCCTGGAGCGATGGTACGCCATCACCTTCGCCATGCGCCTGGATAGGAAGATCCGCCTCA AATCAGCAGCTATGCCAAGGTCAGCATCTGCCTGCCAATGGACACCGACACCCCTCTTGCACTCGCATAC ATTGTCCTCGTTCTGCTCCAATGTTGTTGCCTTTGTTGTCGTCTGTTCCTGCTATGTGAAGATCTACA TCACGGTCCGAAATCCCCAGTACAACCCTCGAGATAAAGACACCAAGATTGCCAAGAGGGATGGCTGTTT GATCTTCACTGACTTCATGTGCATGGCGCCCCATCTCCTTCTATGCGCTGTCGGCACTTATGAACAAGCCT  $\tt CTAATCACTGTTACTAACTCCAAAATCTTGTTGGTTCTCTTCTACCCCCTCAACTCCTGTGCCAATCCGT$  ${\tt TTCTCTATGCTATTTTCACCAAGGCCTTCCAGAGGGACGTGTTCATCCTGCTCAGCAAGTTTGGCATCTG}$ CAAACGCCAGGCCCAGGCCTATCAGGGTCAGAGAGTCTGTCCCAACAATAGCACTGGTATTCAGATCCAA AAGATTCCCCAGGACACGAGGCAGAGTCTCCCCAACATGCAAGATACCTATGAACTGCTTGGAAACTCCC AGCTAGCTCCAAAACTGCAGGGACAAATCTCAGAAGAGTATAAGCAAACAGCCTTGTAAAGGAAAGGCTA ACATAGGTTCATGCAGGTGATGATTCATAGGGTCAGAGTTCATCTCTAGAAAGTATTGCCTC (SEQ ID NO:1)

# FIGURE 1A

MRPGSLLLLVLLLALSRSLRGKECASPPCECHQEDDFRVTCKELHRIPSLPPSTQTLKLIETHLKTIPSLAFSSLPN ISRIYLSIDATLQRLEPHSFYNLSKMTHIEIRNTRSLTYIDPDALTELPLLKFLGIFNTGLRIFPDLTKIYSTDIFF ILEITDNPYMTSVPENAFQGLCNETLTLKLYNNGFTSVQGHAFNGTKLDAVYLNKNKYLTAIDNDAFGGVYSGPTLL DVSSTSVTALPSKGLEHLKELIAKDTWTLKKLPLSLSFLHLTRADLSYPSHCCAFKNQKKIRGILESLMCNESSIRN LRQRKSVNILRGPIYQEYEEDPGDNSVGYKQNSKFQESPSNSHYYVFFEEQEDEVVGFGQELKNPQEETLQAFESHY DYTVCGDNEDMVCTPKSDEFNPCEDIMGYRFLRIVVWFVSLLALLGNIFVLLILLTSHYKLTVPRFLMCNLAFADFC MGVYLLLIASVDLYTHSEYYNHAIDWQTGPGCNTAGFFTVFASELSVYTLTVITLERWYAITFAMRLDRKIRLRHAY TIMAGGWVSCFLLALLPMVGISSYAKVSICLPMDTDTPLALAYIVLVLLLNVVAFVVVCSCYVKIYITVRNPQYNPR DKDTKIAKRMAVLIFTDFMCMAPISFYALSALMNKPLITVTNSKILLVLFYPLNSCANPFLYAIFTKAFQRDVFILL SKFGICKRQAQAYQGQRVCPNNSTGIQIQKIPQDTRQSLPNMQDTYELLGNSQLAPKLQGQISEEYKQTAL (SEQ ID NO:2)

FIGURE 1B

### <u>underlined</u> = deleted in targeting construct

[] = sequence flanking Neo insert in targeting construct

[CAGCGTCAGACGCAGGGCACTGAGAATGTGCGACAGCGCGCAACGATGAAGTAGCCCAG AGGGTCCCTTGGAAAATGAGGCCAGGGTCCC] TGCTGCTGCTTGTTCTGCTGCTCGCCCT GTCCAGGAGCCTGCGGGGCAAAGAGTGTGCGTCTCCACCCTGTGA [GTGTCACCAGGAGG ACGACTTCAGAGTCACCTGCAAGGAGCTCCACCGAATCCCCAGCCTGCCGCCCAGCACCC AGACTCT]GAAGCTCATCGAGACTCATCTGAAGACCATACCCAGTCTTGCATTTTCGAGT CTGCCCAATATTTCCAGGATCTATTTATCTATAGATGCAACTCTGCAGCGGCTGGAACCA CATTCTTTCTACAATTTGAGTAAAATGACTCACATAGAAATCCGGAACACCAGAAGCTTA ACCTATATAGACCCTGATGCCTTGACAGAGCTCCCCTTGCTCAAGTTTCTTGGCATTTTC AATACTGGACTTAGAATATTCCCTGACTTGACCAAAATTTATTCCACGGACATATTCTTT ATACTTGAAATCACAGACAACCCTTACATGACTTCGGTCCCTGAAAACGCATTCCAGGGC CTATGCAATGAAACCTTGACCCTGAAACTGTACAACAATGGATTTACTTCAGTCCAAGGA GCTATAGACAACGATGCCTTTGGAGGAGTATACAGTGGACCAACTTTGCTAGATGTGTCT TCCACCAGCGTCACTGCCCTTCCTTCCAAAGGCCTGGAGCACCTCAAAGAACTGATCGCA AAAGACACCTGGACTCTCAAAAAGCTCCCGCTGTCGTTGAGTTTCCTCCACCTCACTCGG GCTGACCTCTCTTACCCGAGCCACTGCTGCGCTTTTAAGAACCAGAAGAAAATCAGGGGA ATCCTGGAGTCTTTGATGTGTAATGAGAGCAGTATCCGGAACCTTCGTCAAAGGAAATCA GTGAACATCTTGAGGGGTCCCATCTACCAGGAATATGAAGAAGATCCGGGTGACAACAGT GTTGGGTACAAACAAAACTCCAAGTTCCAGGAGAGCCCAAGCAACTCTCACTATTACGTC TTCTTTGAAGAACAAGAGGATGAGGTCGTTGGTTTCGGCCAAGAGCTCAAAAATCCTCAG GAAGAGACTCTCCAAGCCTTCGAGAGCCACTATGACTACACGGTGTGTGGGGACAACGAG GACATGGTGTGTACCCCCAAGTCGGACGAGTTTAACCCCTGTGAAGATATCATGGGCTAC AGGTTCCTGAGAATCGTGGTGTGTTTGTCAGTCTGCTGGCTCTCCTGGGCAATATCTTC GTCCTGCTCATTCTGCTAACCAGCCACTACAAATTGACCGTGCCGCGGTTCCTCATGTGC AACTTGGCCTTTGCAGATTTCTGCATGGGGGTATACCTGCTTCTCATTGCCTCTGTAGAC CTGTACACACTCTGAGTACTACAACCACGCCATCGACTGGCAGACGGGCCCTGGGTGC AACACGGCTGGCTTCTTCACTGTTTTCGCCAGTGAGTTATCAGTGTACACACTGACGGTC ATCACCCTGGAGCGATGGTACGCCATCACCTTCGCCATGCGCCTGGATAGGAAGATCCGC GACACCCCTCTTGCACTCGCATACATTGTCCTCGTTCTGCTGCTCAATGTTGTTGCCTTT GTTGTCGTCTGTTCCTGCTATGTGAAGATCTACATCACGGTCCGAAATCCCCAGTACAAC CCTCGAGATAAAGACACCAAGATTGCCAAGAGGATGGCTGTGTTGATCTTCACTGACTTC ATGTGCATGGCGCCCATCTCCTTCTATGCGCTGTCGGCACTTATGAACAAGCCTCTAATC ACTGTTACTAACTCCAAAATCTTGTTGGTTCTCTTCTACCCCCTCAACTCCTGTGCCAAT CCGTTTCTCTATGCTATTTTCACCAAGGCCTTCCAGAGGGACGTGTTCATCCTGCTCAGC AAGTTTGGCATCTGCAAACGCCAGGCCCAGGCCTATCAGGGTCAGAGAGTCTGTCCCAAC AATAGCACTGGTATTCAGATCCAAAAGATTCCCCAGGACACGAGGCAGAGTCTCCCCAAC ATCTCAGAAGAGTATAAGCAAACAGCCTTGTAAAGGAAAGGCTACGCTAGTCACAGTGAG ACTTACAAAAGGCTGGTTTCTTGAACATGCGTTCCAGTCCCGTGACATGTGAACACATAG GTTCATGCAGGTGATGATTCATAGGGTCAGAGTTCATCTCTAGAAAGTATTGCCTC

#### **FIGURE 2A**

Gene Sequence Structure

91 bp Sequence Deleted

163 bp

Size of full-length
cDNA: 2512 bp

# Targeting Vector\* (genomic sequence)

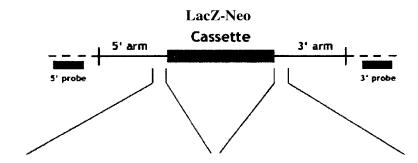
Arm Length:

**5**': 5 kb

3': 1.3 kb

Targeting Vector
- - - - Endogenous Locus

\* Not drawn to scale



5'>ACTTGAGAGCCTCTCCTTCCC CCTCTCCAGCGTGCTCTCCAGCGA TGAGGTCACAGCCCCTCGGAGCCC TCCTCCTCCCTCCCTTCCCCTCCT GCACCCGGGTCTCTTCCAGCGTCA GACGCAGGGCACTGAGAATGTGGC GACAGCGCCAACGATGAAGTAGC CCAGAGGGTCCCTTGGAAAAATGAG GCCAGGGTCCC<3' (SEQ ID NO:3)

FIGURE 2B

Testes +	ا منصبهاما	Friday	Weight	6	(A)								0.181								_	0.076
Heart/	200	, S	Weight	(%)		0.094	0.719	0.105	20.4	0.559	1000	0.030	0.570	5	0.499	0.610		0.455	0.452	100	0.478	0.520
	Hoor	3	Weight	) E	) S	0.100	0.122	0 110		0.133	707	0.12	0.137		0.045	0.051	2000	0.003	0.053	0900	0.000	0.042
Thymus/	500	ב ו	Weight	(%)	2000	0.307	0.354	0.024	7.77	0.265	746.0	70.0	0.258	) .	0.111	0.048	9900	0.200	0.290	0.70	0.213	0.012
	Pymyis	2	Weight	(5)	600.0	7000	0.000	0.055	9	0.063	0800	0.00	0.062		0.010	0.004	000	0.03	0.034	0.035	0.00	0.001
Kidney/	Body		Weight	(%)	1 464	<u>.</u>	1.297	1 393	)	1.278	1.052	700.	1.339	070	1.219	1.316	1 001	50.	1.142	1 16.4	-	1.177
	Kidnev		Weight	(D)	0 327	0.05	0.220	0.342		0.304	0.238	9.5	0.322	077	0.7.0	0.110	0.127	7	0.134	0.146	2	0.095
Liver/	Bodv	`.	Weight	%	5 623	770.0	5.307	5.654		5.010	5 624	1	5.591	7 040	4.0	4.569	5 034	50.0	5.676	6 202	101.0	4.535
Spleen/ Liver/	Liver		Weight	(b)	1 256		0.300	1.388		1.192	1272		1.344	3070	0.433	0.382	0.586		0.666	0.778		0.366
Spleen/	Body	147.2.2.4.1	weignt	8	0.425	)	0.307	0.281	0	0.340	0.354		0.300	0000	0.00	0.191	0.137	5	0.153	0.191	- 1	0.087
	Ë	1	Ξ		않	2	Ŋ	9	3	Ξ	0		Ŋ	α	2	9	9	•	$\infty$	4		_
	Body	Moioh	weigni	(B)	22.339	0	16.960	24.550	007	23.732	22.619	0,0	24.040	9000	0.020	8.360	11.640		11./33	12.545	0	8.070
		dtydo	י בווחווו	(cm)	10	C	8.25	9.5	1	9.7	8.5	•	ဢ	7 5	!	_	∞	1	٥./	80	1	_
		ΔΩ	בי	(days)	48	•	48	48	9	40	48	,	φ	47	: :	φ	48	,	48	48	9	<b>o</b>
	Body Splee	Gender			Female	- Como -	remale	+/+ Maie	Mala	ואמני	Female	Linio	Male	Female		remale	Female	Moto	Maic	Male	And	Na M
					<b>+</b> /+	+/+	+ . F	+/+	+/+		<b>+</b>	7	<b>-</b>	-/-		-/-	<u></u>	,	<u>,</u>	+	,	<u>'</u>

Testes + Epididymis	Weight	/81		0.35	0.387		0.401	0.389
Heart/ Body	Veight (%)	0.5756	0.4826	0.3975	0.4717	0.5572	0.4343	0.4038
Heart	Weight \ (a)	0.145	0.136	0.219	0.201	0.109	0.111	0.102
Fhymus/ Body		1						
Thymus	Weight (a)	0.039	0.043	0.074	0.052	0.040	0.025	0.027
Kidney/ Body	Weight (%)	1.4013	1.3591	1.2598	1.1382	0.9406	1.3304	1.1717
Kidney	Weight (a)	0.353	0.383	0.694	0.485	0.184	0.340	0.296
Liver/ Body	Weight (%)	5.8592	5.1348	5.9304	5.0313	4.4118	5.4545	4.8767
// Liver/ Ki Liver Body Kidney E	Weight (g)	1.476	1.447	3.267	2.144	0.863	1.394	1.232
Spleer Body	veigh (%)	0.881	0.322	0.330	0.319	0.153	0.301	0.201
Spleen	weignt (g)	0.222	0.091	0.182	0.136	0.030	0.077	0.051
Body	weignt (g)	25.191	28.180	55.089	42.613	19.561	25.557	25.263
4	cengtn (cm)	9.5	9.918	11.025	7	7.978	9.47	9.5
Age at	days)	308	308	308	308	307	307	307
200	Genuer rest Length weight W (days) (cm) (g)	Female	Female	Male	Male	Female	-/- Male	Male
		+/+	+/+	+/+	+/+	-/-	<u>+</u> ·	<del>'</del> -